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The effect of exposure to oestrous ewes on rams' sexual behaviour, plasma testosterone concentration and ability to stimulate ovulation in seasonally anoestrous ewes

H.J.D. Rosa ^{*}, D.T. Juniper, M.J. Bryant

Department of Agriculture, The University of Reading, Whiteknights, P.O. Box, Reading RG6 6AT, UK

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Abstract

Previous research has shown that the proportion of seasonal anoestrous ewes that ovulate in response to the introduction of rams ('ram effect') is dependent upon pheromonal and sexual behavioural stimuli emitted by the rams. Close contact with oestrous ewes is likely to increase the rams' libido and level of testosterone secretion, which in turn has been suggested to positively influence the production of pheromones. Thus, the sexual stimulation of rams could be used to improve the efficacy of the 'ram effect'. In the present experiment, 272 ewes were introduced (1) to rams without recent experience of oestrous ewes, (2) to rams recently exposed to oestrous ewes, (3) and (4) to oestrous ewes and rams with or without recent experience with oestrous ewes, or (5) remained isolated from rams. Serum testosterone concentration of rams was elevated equally ($P < 0.05$) when oestrous or anoestrous were introduced. Exposure to oestrous ewes before or after introduction increased ($P < 0.05$) the various measures of ram sexual behaviour directed towards anoestrous ewes. However, there was no statistical evidence that increased sexual activity resulted in an improvement in the ability of rams to stimulate ovulation in anoestrous ewes. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Sheep; reproductive behaviour; Ram effect; Testosterone; Sexual behaviour

^{*} Corresponding author. Present address: Universidade dos Açores, 9701-851 Angra do Heroísmo, Portugal. Tel.: +351-9520-4540; fax: +351-9533-2605.

E-mail address: hrosa@angra.uac.pt (H.J.D. Rosa).

1. Introduction

The proportion of seasonal anoestrous ewes that ovulate in response to the introduction of rams has long been thought to be influenced by pheromone production by the rams (Watson and Radford, 1960). More recently, the role of the sexual behaviour of the rams as a source of ewe stimulation has also been demonstrated (Perkins and Fitzgerald, 1994). According to some reports (e.g., Sanford et al., 1974; Gonzalez et al., 1991a,b; Perkins et al., 1992), the exposure of rams to oestrous ewes can increase the levels of LH and consequently the testosterone secretion in the ram, which in turn has been suggested to positively influence the production of pheromones (Haynes and Haresign, 1987). The close contact with oestrous ewes also increases the rams' libido (Rodríguez Iglesias et al., 1991). In addition, it has also been shown that, at least to some extent, oestrous ewes have the power to induce ovulation in anoestrous ewes (Muir et al., 1989; O'Callaghan et al., 1994; Zarco et al., 1995). What is more, the sexual behaviour displayed by the rams towards the oestrous ewes provides additional visual cues for the anoestrous ewes. Therefore, the presence of oestrous ewes associated with rams or rams which have recent sexual experience with oestrous ewes will most likely allow anovular ewes to be exposed to enhanced olfactory, tactile and particularly visual stimuli which may increase the potency of the stimulation and improve the efficacy of the 'ram effect'.

The main objectives of this work were, therefore, to determine whether the exposure of rams to oestrous ewes, either during the first hours following introduction to anoestrous ewes or during the previous 48 h, would (1) increase the peripheral testosterone concentration of the rams measured a few hours after the introduction to anoestrous ewes, (2) increase the amount of sexual behaviour displayed by the rams towards the anoestrous ewes and (3) increase the proportion of seasonal anoestrous ewes which ovulate in response to ram introduction.

2. Materials and methods

2.1. Animals

The experiment used 272 experimental ewes (also described as anoestrous ewes) and 32 rams. An additional group of 64 ewes were used as teasers for which they were artificially induced into oestrus (described as oestrous ewes). The experimental ewes were adult Northcountry Mule (Bluefaced Leicester ♀ X Swaledale ♂) which had lambed between 15 and 24 January 1996 and whose lambs had been weaned at the beginning of May 1996. Their mean (S.D.) live weight and condition score (Russel et al., 1969) at the beginning of the study were 75.5 (7.7) kg and 3.4 (0.81), respectively. The rams, 16 Texel and 16 Suffolk, were adults and sexually experienced. Their mean (S.D.) live weight and condition score was 102.8 (17.9) kg and 3.7 (0.64), respectively. Before and during the experimental period, both rams and ewes were kept under conditions of natural day length (latitude 51°27'N).

2.2. Treatments

Rams were blocked by breed and ewes blocked by body weight and body condition score. Rams and ewes were randomly allocated from blocks to one of the five following treatments:

Treatment 1: Sixteen ewes remained in complete isolation from the rams until the end of the experiment (control; C).

Treatment 2: Two rams were introduced to 16 experimental ewes (R).

Treatment 3: Two rams and four oestrous ewes were introduced to 16 experimental ewes (RE).

Treatment 4: Two rams which had been mating with four oestrous ewes for 2 days were introduced to 16 experimental ewes (ER).

Treatment 5: Two rams which had been mating with four oestrous ewes for 2 days plus a group of another four oestrous ewes were introduced to 16 experimental ewes (ERE).

Treatments 2 to 5 were replicated four times (four periods) with 2-week intervals, beginning on 4 July 1996. These four periods are described as A, B, C and D according to the dates of ram introduction, respectively, on 4–7 July, 16–19 July, 30 July–2 August and 13–16 August. Within each period and for the different treatments, the rams were introduced to anoestrous ewes on consecutive days, ordered from treatments 2 to 5.

2.3. Experimental procedures

Before the experiment commenced, the ewes and the rams were kept isolated from the sight and smell of animals of the opposite sex for more than 3 months and this condition was maintained during the experimental period for those animals not yet on test at each particular occasion. The rams were introduced to ewes in a 0.5-ha paddock of permanent rye grass pasture where the animals were kept during the first day (day 1) for the purpose of the behavioural study. Two of these paddocks were available, being occupied on alternate days. On day 2, the animals were moved to larger paddocks (1.8 ha) separated from one another by a distance of about 200 m where they remained for the next 8 days. The rams in treatments ER and ERE had mated oestrous ewes for the 48 h immediately before the introduction to the experimental ewes. The oestrous ewes were induced into oestrus with vaginal sponges impregnated with 60 mg MAP (medroxyprogesterone acetate; Veramix, Upjohn) for 13 days, followed by an intramuscular injection of 500 i.u. PMSG (PMSG, Intervet) at the time of sponge withdrawal. The removal of the sponges occurred 24 or 48 h before the oestrous ewes were required (i.e., ewes to be introduced to rams alone in ER and ERE treatments, or ewes to be introduced to rams and experimental ewes in RE and ERE treatments).

2.4. Data collection

2.4.1. Reproductive behaviour

Data of the sexual behaviour of rams were collected on day 1. Observations were carried out during two periods: 06:00–10:00 and 17:00–20:00 h). Two video cameras,

mounted on observation towers, were used to record in detail and continuously all movements of the rams and the movements of other animals in their immediate proximity. Each camera was dedicated to a particular ram and was operated by a trained person. The filming started as soon as rams were introduced. The recorded tapes were later played in a video recorder–TV set and analyzed in detail. In order to individually identify both rams and ewes at a distance, large numbers were painted on their back and flanks, using distinctive colours for the different treatment groups. The sexual behaviour of rams was quantified by measuring the frequency of a series of behaviour elements by the ram directed towards the ewe that are described by Banks (1964) and Lynch et al. (1992): *sniffing*, *flehmen posture*, *ritualized lateral approach*, *mounting*, *service*.

2.4.2. Testosterone concentration in rams' plasma

Blood samples were collected from rams 1 to 3 days before they had any contact with ewes and after the first 4 h of exposure to the experimental ewes. The testosterone concentration in blood serum was determined for both occasions. The bleedings occurred always within the period 10:00–17:00 h. Five milliliters of blood was collected at 20-min interval through a catheter inserted under local anesthesia in the jugular vein on the day before the first sampling period. The catheter was kept in place until the end of the second bleeding period. The blood was collected into heparinized syringes and immediately transferred to centrifuge tubes cooled on ice, centrifuged for 15 min at 4°C and at 3000 rpm and the plasma recovered by decanting and stored at –20°C until assayed. The rams were bled in individual pens inside a barn. Thus, in the second bleeding period (following ram introduction to experimental ewes), they were removed from the paddocks and separated from the ewes for 7 h.

2.4.3. Ovulatory response of ewes

C ewes were bled every 3 days for the determination of plasma progesterone concentration, throughout the experimental period. The ewes in the other treatments were bled for the same purpose, with samples taken three times with 3-day intervals immediately before ram introduction, and again, four times with 2-day intervals, starting on the third day after ram introduction. Blood samples (5 ml) were taken by jugular venipuncture. All the ewes were injected intramuscularly with 20 mg of progesterone (progesterone; Sigma Aldrich) in 2 ml of peanut oil (peanut oil; Sigma Aldrich) 3 days before the introduction of rams. C ewes were injected at the same time as R ewes in all the four different periods of the experiment. A ewe was considered to have ovulated as a response to ram introduction when the progesterone concentration profile indicated a clear rise from values under 0.5 ng/ml in samples taken before ram introduction to values above 0.5 ng/ml in subsequent samples. In other instances, ovulations were considered to have occurred when progesterone levels were above 0.5 ng/ml in at least two consecutive samples.

2.5. Hormone assay procedures

The concentrations of testosterone in ovine plasma were determined using a single antibody, non-extraction radioimmunoassay method. The antiserum (Sheep 505), raised

in sheep against testosterone-3-carboxymethyloxime conjugated to egg albumen (Land et al., 1982), was used at a final dilution of 1:128,000. Gelatine phosphate buffer (GPB) was used as radioimmunoassay diluent and dextran-coated charcoal (DCC) was used to separate the bound from the free testosterone. The radioactive labeled testosterone (testosterone-3-CMO (2-[¹²⁵I]iodohistamine) in methanol:water, 3:1) was purchased from Amersham-Life Science. The radioimmunoassay procedure was carried out in 2 consecutive days.

2.5.1. Day 1

Testosterone standards made up in triple dilutions in GPB ranged from 600 to 0.82 pg/tube. Two hundred microliters of each standard were added to disposable polystyrene tubes (LP3) in quadruplicate. Plasma samples were diluted at 1:41. Four replicates of a control plasma sample pool were included at the beginning and the end of the assay in order to establish the precision of the assay. Four tubes aimed to determine the non-specific binding (NSB), received 300 μ l of GPB. Another set of four tubes for the estimation of radioactivity binding in the absence of unlabelled hormone (B MAX) received 200 μ l of GPB. Four tubes were reserved to determine the total count of cpm (TC). The standard, NSB and B MAX tubes received 5 μ l of steroid-free ram plasma. In order to remove the steroids, the plasma was incubated overnight at 4°C with activated charcoal (10 mg/ml) followed by ultra-centrifugation at $7000 \times g$ for 60 min and filtration in a 0.2- μ m filter. The diluted antiserum was added in a volume of 100 μ l to all tubes except NSB and TC. All tubes received then 100 μ l of GPB solution containing the radioactive labeled testosterone (6000 cpm approximately) and 8-anilino-1-naphthalenesulfonic acid (ANSA, Sigma) in a dilution of 0.2 mg/ml. The tubes were vortexed and incubated overnight at room temperature.

2.5.2. Day 2

The separation of antibody-bound from free testosterone was achieved by absorbing the free steroids to DCC. For this purpose, 200 μ l of an ice-cold suspension of DCC in assay buffer were added rapidly to all tubes except the TC, also previously ice-cooled. The tubes were mixed and incubated in an ice-bath for 15 min. The separation was completed by centrifugation at $2000 \times g$ for 10 min at 4°C. The supernatant, containing the antibody-bound testosterone, was removed by aspiration and radioactivity in the precipitates counted for 60 s in an LKB gamma counter.

The cross-reaction of the antiserum with other steroids is reported by Webb et al. (1985). The amounts of testosterone standard required to inhibit the binding of the radioactive labeled testosterone to the antiserum by 20%, 50% and 80% were 12.9 ± 0.59 , 60.30 ± 2.55 and 341.73 ± 1.77 pg/tube, respectively. Values are means \pm S.E.M. The mean within-assay coefficient of variation calculated from one pooled control sample (10.9 ± 0.26 , S.E.M., ng/ml) at the beginning and the end of the assays was 9.6%. The between-assay coefficient of variation was 12.1%.

Plasma progesterone concentration was determined using the direct-addition enzyme-linked immunoassay (ELISA) described by Sauer et al. (1986) with some modifications for use with ovine plasma. The progesterone antiserum used (S1509/16; Groves et al., 1990) was raised in sheep against progesterone 11-hemisuccinate-BSA (bovine serum

albumin) and was diluted to 1:4000 in 0.17 mM sodium acetate buffer (pH 5.0). The cross-reactions of the antiserum with other different steroids is reported by Groves et al. (1990). The detection limit of the assay was 0.2 ng/ml. The mean intra-assay coefficients of variation calculated from QC₁ (0.4 ± 0.03 ng/ml) and QC₂ (2.8 ± 0.11 ng/ml) in 25 assays were 12.0% and 16.8%, respectively. The inter-assays coefficients of variation calculated from the same quality control samples were 15.3% and 20.9%.

2.6. Statistical analysis

The effect of introduction of ewes on plasma testosterone response of rams within each treatment was tested by Wilcoxon paired-sample test. Kruskal–Wallis analysis was performed to compare the differences among treatments in the variations of testosterone levels. Because data of behaviour also did not follow a normal distribution, square root transformation was applied before analysis. When data contained many zeros, the formula $\sqrt{(0.5 + x)}$ where x represents the original data, was used (Zar, 1996). Data of frequency of sexual behaviour components displayed by the rams were analyzed by multiway factorial analysis of variance (ANOVA), including treatments and date of ram introduction as factors. When ANOVA detected significant differences within factors, multiple comparisons were performed using Fisher's PLSD (post-hoc protected least-squares difference) test. The effect of treatments and date of ram introduction upon the proportion of ewes ovulating was tested by χ^2 analysis, after data have been arranged in contingency tables. In the cases of 2×2 contingency tables, Yates's correction for continuity was applied.

3. Results

Non-parametric analyses and square root transformations were performed in order to analyse data of testosterone concentration and sexual behaviour which did not follow normal distributions. However, for clarity, the original data are presented in tables and figures as means (\pm S.E.M.). The mean testosterone concentration increased significantly ($P < 0.05$) between the two sampling periods in all treatments (Fig. 1). The rises were on average more accentuated in RE (56.3%) and ER (52.4%) treatments, followed by R (36.7%) and ERE (26.8%). However, the differences observed among the treatments did not differ significantly. On both sampling occasions, the mean testosterone concentration was significantly lower ($P < 0.05$) in the first period of ram introduction (A: mean = 3.1 ± 0.6 and 4.8 ± 0.7 ng/ml) than in later periods (B: mean = 8.0 ± 2.3 and 12.6 ± 3.3 ng/ml; C: mean = 8.6 ± 2.3 and 12.6 ± 2.8 ng/ml; D: mean = 12.0 ± 2.0 and 13.7 ± 3.1 ng/ml).

Fig. 2 reports the effect of treatments on the sexual behaviour parameters of the rams towards the anoestrous ewes (time in courtship, investigatory sniffs and ritualized lateral approaches) and towards the oestrous ewes (mounts and services). The flehmen behaviour data refer to the sum of activity directed towards both groups of ewes. Fig. 2 clearly shows a common pattern of variation of the parameters with the treatments, which suggest an appreciable superiority of ER rams over R rams while the RE and

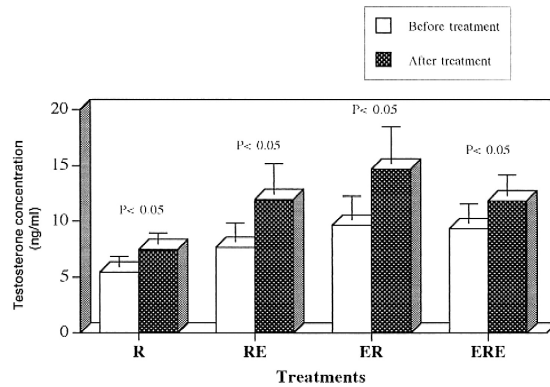


Fig. 1. Mean plasma testosterone concentration in the different groups of rams, before and 4 h after exposure to experimental ewes.

ERE rams are intermediate. The statistical analysis detected significant ($P < 0.05$) differences between treatments in the time spent in courtship and in the observed frequencies of sniffing and lateral approaches. Multiple comparisons tests showed that R rams spent significantly ($P < 0.05$) less time (21 min, 5.0% of total time) courting the experimental ewes than ER rams (82 min, 19.5% of total time) or ERE rams (61 min, 14.5% of total time). R rams also displayed significantly ($P < 0.05$) fewer of sniffs (95)

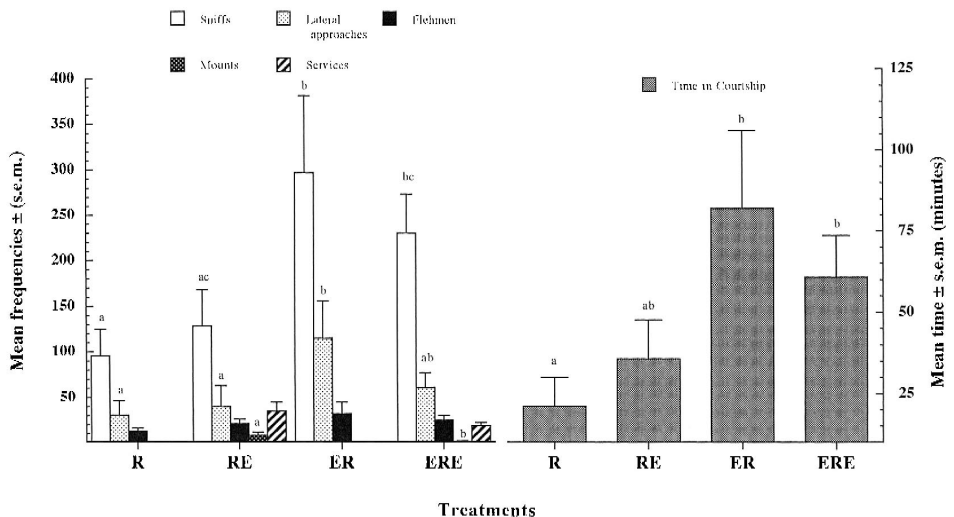


Fig. 2. Effect of treatments on courtship time and on the occurrence of sniffs and lateral approaches displayed by the rams towards experimental ewes and on the occurrence of mounts and services displayed towards oestrous ewes, during the 7-h observation period of the first day of ram introduction. Occurrence of flehmen posture did not discriminate between experimental and oestrous ewes. Different letters within the same parameter indicate significant differences ($P < 0.05$).

towards the experimental ewes than ER (297) and ERE (231) rams and significantly ($P < 0.05$) fewer of lateral approaches (30) than ER rams (115). No significant differences were detected in the time in courtship between ER, RE and ERE rams, but ER rams displayed significantly ($P < 0.05$) more sniffs and lateral approaches than RE rams. In all cases, the best performers were the ER rams which on average dedicated four-fold the time of R rams in courting the experimental ewes and showed four times as many investigatory sniffs and three times as many lateral approaches.

When the total sexual activity of RE and ERE rams was pooled (i.e., sexual activity involved with both experimental and teaser ewes), then R rams dedicated significantly ($P < 0.05$) less time in courting the ewes and displayed significantly ($P < 0.05$) fewer sniffs and lateral approaches than the rams in any other group. On the other hand, the sexual activity of RE, ER and ERE rams was about the same, without any significant difference among them. ER rams displayed more incidences of flehmen, with relatively little difference from rams in treatments RE and ERE but with a large difference from R rams (almost three-fold). However, the large within treatments variation did not allow this difference to be statistically significant ($P = 0.44$).

RE rams mounted significantly ($P < 0.05$) more ewes than ERE rams (8.0 ± 3.7 , range 1–33 vs. 1.3 ± 0.6 , range 0–4) and performed more services (35.1 ± 10 , range 1–93 vs. 18.9 ± 3.4 , range 0–31) although without statistical significance. The small differences between RE and ERE rams in the incidence of the other sexual behaviours directed towards oestrous ewes were not statistically significant, and the time dedicated to courtship of these ewes was the same (8.5% and 9% of the total time) in both treatments.

A trend was observed for poorer performances in the first period (A) of the experiment, followed by superior and similar performance in periods B, C and D, for all behaviour parameters displayed by the rams towards the anoestrous ewes (Fig. 3). The flehmen behaviour, which was directed towards all ewes, also followed this tendency.

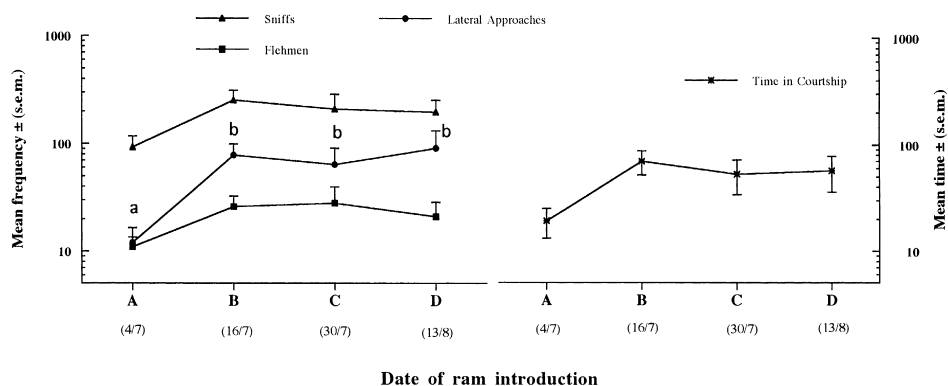


Fig. 3. Effect of date of ram introduction on duration of courting time and on the frequency of courtship components displayed by the rams towards the experimental ewes. Each point represents the mean of the first day of ram introduction (morning + afternoon). Different letters among points indicate significant differences ($P < 0.05$).

Table 1

Effect of treatments and date of ram introduction upon the occurrence of ovulation. Values are percentages and proportions (in parenthesis) of ewes ovulating until day 9 following ram introduction

Treatments	Date of ram introduction				Overall significance	Total
	4/7	16/7	30/7	14/8		
Control	0 (0/16)	0 (0/16)	13 (2/16)	13 (2/16)	n.s.	6 (4/64)
R	47 (7/15)	88 (14/16)	94 (15/16)	94 (15/16)	$P < 0.01$	81 (51/63)
RE	69 (11/16)	100 (16/16)	87 (13/15)	94 (15/16)	$P < 0.01$	87 (55/63)
ER	80 (12/15)	88 (14/16)	94 (15/16)	94 (15/16)	n.s.	89 (56/63)
ERE	69 (11/16)	100 (16/16)	100 (16/16)	87 (13/15)	$P < 0.05$	89 (56/63)
Overall significance	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$		$P < 0.001$

However, the differences in time spent in courtship, the number of sniffs and the number of flehmen failed to reach statistical significance. The number of lateral approaches was significantly ($P < 0.05$) higher in the last three periods (78 ± 21 , 64 ± 27 and 91 ± 41 for periods B, C and A, respectively) when compared to the first period (12 ± 4.5).

Practically all ewes kept isolated from rams remained anovular during the course of the experiment, except 2 ewes which ovulated around days 3–6 August. Of the ewes that were introduced to rams, only two were ovulating at the time of ram introduction. There was an overall effect ($P < 0.001$) of treatments on the incidence of ovulation in all four dates of ram introduction which was also detected when the totals of each treatment were compared (Table 1). However, this effect is due to the virtual absence of ewes ovulating in the control group. In fact, group by group comparisons showed that differences between control ewes and ewes in any other treatment were always significant (at least $P < 0.01$) but significant differences were not detected in comparisons among R, ER, RE and ERE treatments. In the first period of ram introduction, the exposure of rams to anoestrous ewes tended to stimulate more ewes to ovulate (73% for pooled estimation of RE, ER and ERE treatments vs. 47% for R treatment) but differences failed to reach statistical significance ($P = 0.13$). In this period, major differences were observed between R (47%) and ER (80%) ewes but again significance level was not achieved ($P = 0.13$). Date of ram introduction had a significant effect upon the number of ewes ovulating in R, RE and ERE treatments but the results suggest that this effect was mostly due to a weak response of ewes when rams were introduced on 4 July.

4. Discussion

The main objective of this study was to test the hypothesis that the exposure of rams to oestrous ewes, either before or during the introduction to seasonal anoestrous ewes, would increase the circulating testosterone levels and the libido of the rams resulting in an enhancement of the stimulation received by the anoestrous ewes and the consequent

improvement in the effectiveness of the 'ram effect'. The results indicate that the testosterone concentration did not increase significantly and, in spite of the substantial improvement observed in the sexual activity, there was no significant increase in the number of ewes ovulating. Therefore, the hypothesis is not proven. Previous reports by Knight (1985), Knight and Gibb (1990) and Rodríguez Iglesias et al. (1991) have suggested that integrating social facilitation (i.e., the use of oestrous ewes) with the 'ram effect' could result in a more effective response. In this study, due to the high ovulatory response of the ewes in all treatments, including the one with non-stimulated rams (R), there was little scope for the effect of treatments to be detected.

This is surprising, since ewes from breeds of relatively long seasonal anoestrus are not expected to respond in any magnitude to ram introduction until some of them are ovulating spontaneously (Martin et al., 1986). A high proportion of ewes ovulated following exposure to rams in July (4/7 and 16/7) when spontaneous ovulatory activity is absent in this genotype (mule) as has been shown in previous reports by Rekik et al. (1991) and Mitchell et al. (1997) and confirmed here. In the present study, the ewes were subject to a degree of stimulation not usual in normal farm conditions. Firstly, in all treatments the ram:ewe ratio was high (1:8) and the space available for the flock to run during the first day of ram introduction was relatively small (0.5 ha) which may have facilitated the frequency of contacts and the spread of pheromones. Secondly, in all treatments except R, the rams were stimulated with oestrous ewes. Thirdly, the reintroduction of rams in the afternoon, which reactivated the sexual activity of the rams, might have provided additional stimulation. These results together seem to suggest that the proportion of ewes which are supposedly in 'deep' anoestrus and which ovulate as response to ram introduction can be higher than is usually expected if the stimulation provided by the rams is maximized.

The means of testosterone concentration were within the range reported in the literature. The mean testosterone concentration increased significantly in all treatments, despite the fact that R rams were only introduced to non-receptive ewes. Results in many studies indicate rapid rises in testosterone levels when rams and other mammals are introduced to oestrous females (part of the phenomenon known as 'female-effect') but there is a lack of reports on the effect of introduction of rams to non-oestrous ewes. In the study of Gonzalez et al. (1991a) the mean testosterone level of rams increased from 0.88 to 1.39 ng/ml of plasma 3 h after being in contact with non-receptive ewes but the difference was not significant. However, during the same period the LH concentration increased significantly. Considering that these two hormones are closely associated in the ram, it is reasonable to suppose that the lack of significance in the increase of testosterone was due to a sampling period of short duration (3 h). Taken together, the results above and the findings in the present work, strongly suggest that, like oestrous ewes, non-oestrous ewes also have the ability to stimulate the release of testosterone in the ram. On this basis, the concept of 'female effect' should be extended to anoestrous ewes. Lending more support to this idea is the fact that, although little is known about the specific stimuli that elicit the hormonal response, the results of Gonzalez et al. (1991b) have indicated that it is unlikely to originate from olfactory cues exclusive to oestrous ewes as wool, urine and vaginal secretions from sexually receptive ewes placed in a mask did not induce a response. Other stimuli, some of which may also

be released by sexually non-receptive ewes, are, therefore, likely to be involved. Nevertheless, although without statistical significance, the rises in testosterone were more accentuated in rams of treatments comprising oestrous ewes, which suggest a tendency for ewes to be more effective at stimulating the rams when they are sexually receptive.

As expected, the exposure of the rams to oestrous ewes substantially improved their level of sexual activity, measured as the total time spent in courtship and the frequency of courtship components displayed towards all ewes (i.e., oestrous + anoestrous). When no oestrous ewes were present and the rams had no recent sexual experience (treatment R) the rams spent only a limited period of time courting these ewes (5% of total) while in the other treatments the rams extended the period of courtship to 17–23% of total time. When oestrous ewes were present, (treatments RE and ERE), the rams in both treatments dedicated about the same time in courting the oestrous and the anoestrous ewes. In spite of diverting the attention of the rams, the presence of sexually receptive ewes still contributed positively to increase the time spent by the rams in courting the experimental ewes.

A period of 24–48 h of sexual experience prior to introduction also was sufficient to increase the libido of the rams. While the typical R ram's ewe-seeking activity and courtship behaviour was limited to the first moments of joining, the sexually experienced ER ram would be very much more sexually motivated, persistently seeking and following the ewes. This activity was not limited to the first period of introduction to ewes but persisted during the first day of cohabitation.

The rams were sexually less active when they were introduced to ewes in the beginning of July than in subsequent periods. It has been well established that the libido of rams accompanies the seasonal variation of other reproductive characteristics (i.e., gonadotrophins and testosterone secretion, testicular size and semen production) with the peak of sexual activity normally occurring at the end of summer/autumn (Lincoln and Davidson, 1977; Lincoln and Short, 1980; Ortavant et al., 1988). Seasonal variations in sexual activity of as much as 50% were reported by Schanbacher and Lunstra (1976). The difference found in the present case must, therefore, be best explained by a negative effect of the photoperiod in early July. Some support for this assumption is given by the fact that the testosterone levels of the rams in early July were also lower (at the $P < 0.05$ level of significance) than in the other periods.

In conclusion, this study has clearly demonstrated that the exposure of rams to oestrous ewes increased the amount of sexual activity directed towards anoestrous ewes. However, there was no statistical evidence that the increased sexual activity resulted in an increased proportion of anoestrous ewes subsequently ovulating. Peripheral testosterone concentration of rams was elevated equally when oestrous or non-oestrous ewes were introduced.

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